Maternal Vitamin D Concentrations During Pregnancy, Fetal Growth Patterns and Risks of Adverse Birth Outcomes

Kozeta Miliku, MD[1,2,3], **Anna Vinkhuyzen, PhD**[5,6], **Laura M. E. Blanken, MD**[1,4], **John J. McGrath, MD, PhD**[5,6], **Darryl W. Eyles, PhD**[5,6], **Thomas H. Burne, PhD**[5,6], **Albert Hofman, MD, PhD**[2], **Henning Tiemeier, MD, PhD**[2,4], **Eric A.P. Steegers, MD, PhD**[7], **Romy Gaillard, PhD**[1,2,3], and **Vincent W.V. Jaddoe, MD, PhD**[*,1,2,3]

[1]The Generation R Study Group, Erasmus MC, University Medical Center, Rotterdam, the Netherlands [2]Department of Epidemiology, Erasmus MC, University Medical Center, Rotterdam, the Netherlands [3]Department of Paediatrics, Erasmus MC, University Medical Center, Rotterdam, the Netherlands [4]Department of Child and Adolescent Psychiatry, Erasmus MC, University Medical Center, Rotterdam, the Netherlands [5]Queensland Brain Institute, The University of Queensland, Brisbane, Australia [6]Queensland Centre for Mental Health Research, Park Centre for Mental Health, Wacol, Australia [7]Department of Obstetrics and Gynaecology, Erasmus MC, University Medical Center, Rotterdam, the Netherlands

Abstract

Background—Maternal vitamin D deficiency during pregnancy may impact fetal outcomes.

Objective—To examine whether maternal 25-hydroxyvitamin D (25(OH)D) concentrations in pregnancy affect fetal growth patterns and birth outcomes.

Design—Population-based prospective cohort in Rotterdam, the Netherlands among 7098 mothers and their offspring. We measured 25(OH)D concentrations at a median gestational age of 20.3 weeks (range 18.5-23.3 weeks). Vitamin D concentrations were analyzed continuously and in

[*]Corresponding author: V.W.V. Jaddoe, MD, PhD, The Generation R Study Group (Na-2915), Erasmus MC, University Medical Center, PO Box 2040, 3000 CA Rotterdam, The Netherlands; Phone: +31 (0) 10 7043405, Fax: +31 (0) 10 4089382 ; v.jaddoe@erasmusmc.nl.

quartiles. Fetal head circumference, length and weight estimated by repeated ultrasounds, preterm birth (gestational age <37 weeks) and small-size for gestational age (<5th percentile).

Results—Adjusted multivariate regression analyses showed that as compared to mothers with second trimester 25(OH)D concentrations in the highest quartile, those with 25(OH)D concentrations in the lower quartiles had offspring with third trimester fetal growth restriction leading to a smaller birth head circumference, shorter birth length and lower weight at birth (all *P* values <0.05). Mothers who had 25(OH)D concentrations in the lowest quartile had increased risks of preterm delivery (Odds Ratio (OR) 1.72, 95% Confidence Interval (CI) 1.14, 2.60) and small-size for gestational age children (OR 2.07, 95% CI 1.33, 3.22). The estimated population attributable risks of 25(OH)D concentrations <50 nmol/L for preterm birth or small-size for gestational age were 17.3% and 22.6%, respectively. The observed associations were not based on extreme 25(OH)D deficiency, but present within the common ranges.

Conclusions—Low maternal 25(OH)D concentrations are associated with proportional fetal growth restriction and with increased risks of preterm birth and small-size for gestational age at birth. Further studies are needed to investigate the causality of these associations and the potential for public health interventions.

Introduction

Vitamin D deficiency is common and related to various non-communicable disease (1). An accumulating body of evidence suggest that vitamin D is also crucial for fetal development because of its important role during cell proliferation, differentiation and maturation processes (2, 3). Suboptimal vitamin D concentrations may affect early organogenesis and subsequently affect later health and disease (4). Also, vitamin D is important for placental function, calcium homeostasis, and bone mineralisation, which are all important determinants for fetal growth and development (5),(6). Thus far, most published studies focused on the associations of maternal vitamin D status during pregnancy with fetal development were mainly based on birth weight and showed inconsistent results (7–9). However, birth weight is just a proxy for fetal growth and development. Different fetal growth patterns and body proportions may lead to the same birth weight. Not much is known about the direct effects of maternal vitamin D status on fetal growth and development patterns in healthy populations (8, 10–13). The inconsistent results from previous studies may be explained by differences between study populations. Because ethnic differences in both vitamin D concentrations and birth outcomes have been reported, the effects of maternal vitamin D status on fetal outcomes may differ between specific populations (14, 15). Based on previous studies suggesting that low vitamin D concentrations, may lead to suboptimal placentation and fetal skeletal growth, we hypothesized that low vitamin D concentrations may lead to fetal growth restriction and increased risks of adverse birth outcomes.

Therefore, we examined in a multi-ethnic population-based prospective cohort study among 7098 mother-offspring pairs, the associations of circulating 25-hydroxyvitamin D (25(OH)D) concentrations with repeatedly measured fetal growth characteristics during second and third trimester and the risks of adverse birth outcomes. Additionally, we

explored whether adverse birth outcomes were associated with cord blood 25(OH)D concentrations.

Methods

Design and Study Population

This study was embedded in the Generation R Study, a population-based prospective cohort study from fetal life onwards in Rotterdam, the Netherlands (16). All children were born between April 2002 and January 2006. Enrolment in the study was aimed at early pregnancy, but was allowed until the birth of the child. The study protocol was approved by the local Medical Ethical Committee. Written consent was obtained from all participating mothers. Second trimester 25(OH)D concentrations were measured in 7176 mothers. For the present study, we excluded pregnancies leading to twin births (n = 77) and loss to follow-up at birth (n = 1). Thus, the cohort for analysis comprised 7098 mothers with any fetal or birth outcome available. (Supplementary Figure 1. Flowchart).

Maternal and Cord 25(OH)D Blood Concentrations

Maternal venous blood samples were collected in second trimester (median 20.3 weeks of gestation, range 18.5-23.3 weeks), whereas umbilical cord blood samples were collected immediately after delivery (median 40.1 weeks of gestation, range 35.9-42.3 weeks). Cord blood vitamin D concentrations represent neonatal vitamin D status at birth. Measurements of 25(OH)D concentrations were conducted at the Eyles Laboratory at the Queensland Brain Institute, University of Queensland, Australia, in 2014.

Total 25(OH)D was calculated as the sum of $25(OH)D_2$ and $25(OH)D_3$ measured in plasma as previously described (17). Samples were quantified using isotope dilution liquid chromatography-tandem mass spectrometry. Linearity of 25(OH)D concentration was assessed using matrix-matched calibration standards, with R2 values of >0.99 across the calibration range (10 – 125 nmol/L). Inter-assay inaccuracy and imprecision were assessed at four concentration levels for $25(OH)D_3$ (48.3, 49.4, 76.4, 139.2 nmol/L) and a single level (32.3 nmol/L) for $25(OH)D_2$ using certified reference materials and were excellent at all concentration levels tested. Inter-assay inaccuracy and imprecision were both <10% for $25(OH)D_3$ and <17% for $25(OH)D_2$, respectively. We categorized vitamin D status into quartiles (quartile 1: median (full range) 14.7 nmol/L (1.5 to 24.1); quartile 2: 35.1 nmol/L (24.2 to 46.6); quartile 3: 59.0 nmol/L (46.7 to 73.7); quartile 4: 91.6 nmol/L (73.8 to 193.2)). Since optimal vitamin D concentrations remain a subject of debate (18, 19), we performed a sensitivity analysis by using cut-offs concentrations according to previously used cut-offs and recommendations (severely deficient: <25.0 nmol/L; deficient: 25.0 to 49.9 nmol/L; sufficient: 50.0 to 74.9 nmol/L; optimal   75.0 nmol/L) (18, 20–24).

Fetal Growth Measurements

Fetal ultrasound examinations were carried out in two dedicated research centers in second (median 20.5 weeks of gestation, 95% range 18.6–24.3) and third trimester (median 30.3 weeks of gestation, 95% range 28.4–32.8) (25). The first trimester ultrasound was primarily used for establishing gestational age (26). In second and third trimester, we measured fetal

head circumference, abdominal circumference and femur length to the nearest millimeter using standardized ultrasound procedures (27). Estimated fetal weight was subsequently calculated using the formula of Hadlock et al (28). Longitudinal growth curves and gestational-age-adjusted standard deviation scores (SDS) were constructed for all fetal growth measurements (26). These gestational-age-adjusted SDS were based on reference growth curves from the whole study population, and represent the equivalent of z-scores (26).

Birth Outcomes

We obtained information about offspring sex, gestational age, head circumference, length and weight at birth from medical records (29). Because head circumference and length were not routinely measured at birth in each delivery center, fewer measurements were available (n = 3681 and n = 4533 for head circumference and length at birth, respectively), as compared to birth weight. Gestational-age-adjusted SDS for head circumference, length and weight were constructed using North European growth standards (30). We defined preterm birth as a gestational age of <37 weeks at birth, low birth weight as a birthweight <2500 grams, and small-size for gestational age at birth as a gestational-age-adjusted birthweight below the 5th percentile (-1.79 SD).

Covariates

We used questionnaires at enrollment in the study to collect information about maternal age, ethnicity, educational level, parity, the presence of anorexia, smoking, alcohol usage, folic acid and vitamins supplementation (29). Maternal energy, iron, zinc and calcium dietary intake during pregnancy was measured at enrollment with a validated semi-quantitative food frequency questionnaire (FFQ) (31). Ethnicity and educational level were defined according to the demographic classification of Statistics Netherlands (32, 33). Ethnicity was categorized in the following groups: European, Cape Verdean, Dutch Antillean, Moroccan, Surinamese, Turkish and Others. We measured maternal height and weight at enrollment and calculated body mass index (kg/m^2). Information about gestational hypertensive disorders (gestational hypertension, preeclampsia) and gestational diabetes was available from medical records (34). The date of blood sampling was categorized into summer, fall, winter, and spring, based on the European seasons.

Statistical Analysis

First, we performed regression analyses to relate second trimester maternal 25(OH)D concentrations with fetal growth characteristics in second, and third trimester separately. Second, we assessed the associations of maternal 25(OH)D concentrations with longitudinally measured of fetal head circumference, fetal femur length and body length at birth, and fetal weight, expressed as SDS, using unbalanced repeated measurement regression analyses. These analyses enable optimal use of available data, taking into account correlations within subjects and assessing both time dependent and independent associations (35). Since body length can not be estimated during fetal life, we used femur length in second and third trimester and body length at birth to assess length growth. We measured femur length to the nearest millimeter using standardized ultrasound procedures (27). For weight we used estimated fetal weight in second and third trimester and birth weight. Third,

we used logistic regression models to assess the associations of maternal 25(OH)D concentrations with the risks of preterm birth, low birth weight, and small-size for gestational age. For all the analyses, 25(OH)D concentrations were analysed both continuously and by using quartiles. As sensitivity analysis, we also analysed 25(OH)D concentrations in groups of previously used and recommended cut-offs instead of quartiles (18, 20–24). We calculated the population attributable risk (PAR) for these outcomes as: PAR% = [(Rate$_{total\ population}$ − Rate$_{unexposed}$)/(Rate$_{total\ population}$)]*100%. Finally, we explored the associations of birth characteristics with cord blood 25(OH)D concentrations using linear regression models. All regression models were first adjusted for season when blood samples were drawn and maternal ethnicity (Basic models), and subsequently additionally for maternal age, education, parity, body mass index at enrolment, smoking, alcohol use, the presence of anorexia, folic acid and vitamins supplement use and energy, iron, zinc and calcium dietary intake during pregnancy, gestational hypertensive disorders and gestational diabetes (Adjusted models). These covariates were included in the models based on their associations on fetal and birth outcomes in previous studies, or a change in effect estimates of >10%. Adding maternal nutritional data (iron, zinc and calcium dietary intake and vitamin supplements) did not materially change the effect estimates but slightly improved the model fit (R^2, -2log Likelihood and the Nagelkerk R^2 values). Because of the strong associations of ethnicity with both 25(OH)D concentrations with and fetal outcomes, we first adjusted the regression models for maternal ethnicity and second, we restricted the analyses to Europeans only, the largest ethnic subgroup in our cohort (36, 37). A *P value* of <0.05 was considered as statistically significant. To adjust for multiple testing in the analyses of adverse birth outcomes with cord blood 25(OH)D concentrations, we applied Bonferroni correction considering a *P value* <0.025 (0.05/2) as significant. To diminish potential bias associated with attrition, missing values of covariates, were multiple imputed by generating 5 independent datasets using the Markov Chain Monte Carlo (MCMC) method. The multiple imputation procedure was based on the correlation between each variable with missing values and the other subject characteristics (38, 39). Statistical analyses were performed using SPSS version 21.0 (IBM SPSS Statistics for Windows, Armonk, NY: IBM Corp). Subject characteristics before and after imputation, including the percentage of missing values are given in the Supplementary Table 1. The unbalanced repeated-measurements analysis, including the Prox Mixed module, was performed with the Statistical Analysis System (version 9.3; SAS Institute Inc, Cary NC).

Results

Subject Characteristics

Table 1 shows that the median value and 95% range of maternal 25(OH)D is 46.7 (7.0, 119.4) nmol/L. Maternal and cord blood 25(OH)D concentrations were correlated (r=0.62, *P value* <0.01). Growth characteristics during second and third trimester and at birth are shown in Table 2. Supplementary Table 2 gives the subject characteristics for the each quartile of 25(OH)D concentrations. We observed that mothers with a Turkish or Moroccan ethnicity had the lowest 25(OH)D concentrations.

Maternal 25(OH)D Concentrations and Fetal Growth Characteristics

Figure 1 shows the associations of maternal 25(OH)D concentrations during pregnancy with longitudinally measured fetal head circumference, length and weight. As compared to mothers with 25(OH)D concentrations in the highest quartile (range 73.8 to 193.2 nmol/L), those with 25(OH)D concentrations in the first (range 1.5 to 24.1 nmol/L) and second (range 24.2 to 46.6 nmol/L) quartiles had offspring with restricted fetal head circumference growth from second trimester onwards, leading to a smaller head circumference at birth (differences: -0.20 SDS 95% Confidence Interval (CI) (-0.29, -0.12) and -0.09 SDS 95% CI (-0.17, -0.01) for first and second quartile of 25(OH)D concentrations, respectively). The associations of second trimester maternal 25(OH)D concentrations with fetal length growth and weight growth tended to be similar as those observed for head circumference. Results from the regression models focused on the associations of 25(OH)D concentrations with one fetal growth measure at each time point showed similar results (Supplementary Table 3). We observed similar associations when using vitamin D cut-offs according to previously used clinical cut-offs and recommendations (Supplementary Table 4). Also, although we observed tendencies for similar effect estimates when we restricted the analyses to Europeans only (N = 4069), not all associations reached statistical significance due to smaller sample sizes (Supplementary Table 5).

Maternal 25(OH)D Concentrations and Risks of Adverse Birth Outcomes

Figure 2 shows that as compared to mothers who had 25(OH)D concentrations in the highest quartile (range 73.8 to 193.2 nmol/L), those who were in the lowest quartile of 25(OH)D concentrations (range 1.5 to 24.1 nmol/L) had increased risks of children born preterm birth (Odds Ratio (OR) 1.72 (95% CI 1.14, 2.60)), with a low birth weight (OR 1.56 (95% CI 1.02, 2.39) and with a small-size for gestational age at birth (OR 2.07 (95% CI 1.33, 3.22)). We observed dose-response associations suggesting that higher maternal 25(OH)D concentrations across the full range were associated with lower risks of preterm birth, low birth weight and small-size for gestational age at birth (all *P values* <0.05). We observed tendencies for similar associations when using 25(OH)D cut-offs according to previously used clinical cut-offs and recommendations instead of quartiles (Supplementary Figure 2). Similar effect estimates were observed when we restricted our analyses to European subjects only, but not all associations were significant (Supplementary Figure 3). The estimated population attributable risks of vitamin D concentrations <50 nmol/L for preterm birth or low birth weight or for small-size for gestational age in our population were 17.3%, 18.4% and 22.6%, respectively.

Adverse Birth Outcomes and Cord Blood 25(OH)D Concentrations

Table 3 shows that higher weight at birth and gestational age adjusted birth weight were associated with higher cord blood 25(OH)D concentrations (differences 0.04 SDS (95% CI 0.01, 0.07)) and 0.03 SDS (95% CI 0.01, 0.06) per 1 SD increase in birth weight and gestational age adjusted birth weight, respectively). The association of higher gestational age at birth with cord blood 25(OH)D concentrations was of borderline significance (difference -0.01 SDS (95% CI -0.03, 0) per 1SD increase in gestational age. Tendencies for similar

effect estimates were observed when we restricted our analyses to European subjects only (Supplementary Table 6).

Discussion

Results from this large population-based prospective cohort study suggest that second trimester lower maternal 25(OH)D concentrations are associated with third trimester fetal head, length, and weight growth restriction and with increased risks of preterm birth, low birth weight and small-size for gestational age at birth. These associations were not restricted to the extremes, but tended to be present across the full spectrum of maternal 25(OH)D concentrations. Also, a smaller size at birth was associated with lower cord blood 25(OH)D concentrations.

We hypothesized that suboptimal maternal 25(OH)D concentrations affect fetal development leading to an increased risk for adverse birth outcomes. Previous studies reported inconsistent results on the associations of maternal 25(OH)D concentrations with birth outcomes (2, 7–9, 40). A previous study among 2473 mother-children pairs, in a multi-center cohort in the United States reported that low concentrations of maternal 25(OH)D are associated with low weight at birth (9). In line with these findings, a study among 2146 mother-children pairs in the United States observed that maternal 25(OH)D concentrations were positively associated with weight at birth (40). Results from another population-based cohort study in the Netherlands among 3730 mother-children pairs, also suggested that lower maternal 25(OH)D concentrations are associated with lower birth weight (7). A recent meta-analysis of randomized controlled trials suggested that vitamin D supplementation during pregnancy was associated with increased circulating 25(OH)D concentrations and a higher birth weight (41). Thus, altogether previous studies suggest that higher vitamin D concentrations in pregnancy lead to higher birth weight.

Using birth weight as main fetal outcome has strong limitations. Birth weight does not give information about longitudinal fetal growth and development patterns and fetal body proportions. We examined the impact of maternal 25(OH)D concentrations on head circumference, length and estimated fetal weight during second and third trimester of pregnancy and at birth. We used repeatedly and directly measured fetal growth characteristics and observed that lower maternal 25(OH)D concentrations were associated with restricted fetal head circumference growth from second trimester onwards, correlating with a small head circumference at birth. Similar associations were observed for fetal length growth and fetal weight growth. In line with our findings on head circumference at birth, a multi-center cohort in the United States suggested that lower maternal 25(OH)D concentrations during mid-pregnancy are associated with a smaller head circumference at birth (40). In contrast, a study among 2382 mother-child pairs in Spain, showed that higher maternal 25(OH)D concentrations in early pregnancy are associated with a smaller head circumference at birth (8). Regarding birth length, results from a recent meta-analysis of randomized controlled trials suggested that neonates in the maternal vitamin D supplemented group had a higher birth length compared with the control group (41). In contrast to these findings, a study among 559 mother-children pairs in India, did not observe any association of maternal 25(OH)D concentrations and length at birth (42). These

differences may be due to differences in study populations. Results from the longitudinal analyses of our large study, suggest that low maternal 25(OH)D concentrations are associated with a proportional growth restriction, though the largest effect may be present on fetal length growth.

We observed that lower maternal 25(OH)D concentrations during mid-pregnancy are associated with a higher risk of preterm birth. A previous study among 2382 mother-child pairs in Spain did not observe any association of maternal 25(OH)D concentrations during early pregnancy with the risk of preterm birth (8). These results are not in line with what we observe, this may be due to specific study population, and smaller sample size to detect these associations. Our results regarding the risk of low birth weight and of small-size for gestational age are in line with findings from another cohort study in the Netherlands, which observed that lower maternal 25(OH)D concentrations during early pregnancy are associated with an increased risk of low birth weight and small-size for gestational age (7). Also, a previous study among 3658 Chinese mothers, whose 25(OH)D concentrations were assessed at different time points during pregnancy, observed that lower maternal 25(OH)D concentrations throughout pregnancy elevate the risk of small-size for gestational age at birth (43). The observations linking low maternal vitamin D to both preterm birth, low birth weight and small-size for gestational age at birth are important. All three outcomes are associated with perinatal mortality and later-life chronic disease (44). We also observed that children born preterm or with a small-size at birth have lower cord blood 25(OH)D concentrations. Results from a study in Boston among 471 infants, suggested that preterm born infants have a higher risk of having lower 25(OH)D cord blood concentrations (45). Thus, preterm birth and small size at birth may be both a consequence and a risk factor of vitamin D deficiency. Low vitamin D concentrations in early life may affect health in later life (46, 47).

Form our observational study, it is not possible to establish the causality for the observed associations. However, several biological mechanisms have been suggested linking maternal 25(OH)D concentrations to fetal development. Maternal vitamin D may affect placental vascularisation (48). It has been suggested that vitamin D receptors and $1,25(OH)_2D$ regulate placental secretion of human placental lactogen and other hormones that affect maternal glucose and fatty acid metabolism, which provide energy for fetal needs (45, 48). Further studies are needed focusing on the causality and mechanisms explaining the observed associations are needed.

Our findings are important from a population-based perspective. The upper limit of first 2 quartiles of maternal 25(OH)D concentrations correspond to the value of <50 nmol/L, which is defined as 25(OH)D deficiency (19, 20). In our study, 53% of all mothers had 25(OH)D deficient concentrations, leading to high estimated population attributable risks. In the Netherlands, pregnant women are advised to use folic acid supplements (400 μg/day) prior to and up to the 10-12th week of pregnancy and vitamin D supplements (10 μg/day). Therefore, results from our study support population-strategies to improve vitamin D concentrations in pregnant women.

Some methodological considerations need to be considered. To our knowledge, this is the largest multi-ethnic population-based prospective cohort study focused on the associations of maternal 25(OH)D concentrations with directly measured fetal growth characteristics in different periods of pregnancy. Among mothers with 25(OH)D concentrations available, we had a limited loss to follow-up, therefore we do not expect biased results due to selective follow-up. We used 25(OH)D concentrations, which are the best and most widely used indicator of vitamin D status (49). We analyzed vitamin D concnetrations as continuous data, quartiles and previously used clinical cut-offs (18, 20–24). The cut offs remain subject of debate (18, 19). In line with recommendations of the Endocrine Society and based on previous results from our and other cohort studies, we created four vitamin D groups, including severely deficient (<25.0 nmol/L), deficient (25.0 to 49.9 nmol/L), sufficient (50.0 to 74.9 nmol/L) and optimal (75.0 nmol/L) (18, 20–24). The Institute of Medicine (IOM) defines vitamin D as deficient (<50nmol/L), and sufficient (50nmol/L), which would lead to categorizing our severely deficient and deficient groups as deficient, and categorizing the sufficient and optimal groups as sufficient or adequate. We consider an advantage of our categories as compared to IOM categories that we have more groups and can compare our results with previous pregnancy studies. Also, our findings suggest that the effects of vitamin D on fetal outcomes are not restricted to IOM deficient groups, but present across the full common range. 25(OH)D concentrations were assessed during second trimester and in cord blood at birth. The correlation coefficient between maternal serum 25(OH)D concentrations during second trimester and in cord blood was r=0.62 (*P value* <0.01). This correlation varies between studies. Some studies report a higher degree of correlation, but still report a difference between maternal 25(OH)D concentrations during third trimester of pregnancy and 25(OH)D concentrations in cord blood at birth (50). A limitation of our study may be that we do not have maternal 25(OH)D concentrations measured during first trimester. We measured fetal development by direct second- and third trimester fetal ultrasound examinations. Since the main outcomes were correlated, we did not adjust the main analyses for multiple comparisons. Another limitation of our study is the lacking of detailed information on vitamin D supplementation and on conditions that may influence vitamin D status, such as bariatric surgery or different gastrointestinal diseases. However, other nutritional factors may influence the observed associations. Since previous studies suggested that iron, zinc, calcium and other vitamin supplements influence fetal growth and birth outcomes, we included these nutritional factors in our regression models. Adjustment for these factors did not materially change the effect estimates. Several trials suggested that supplementation of these micronutrients lower the risks of pregnancy complications in women at risk of deficiencies or adverse outcomes (51–56). Because, the intake of these micronutrients was estimated from a FFQ, we may not have the precision in these levels as compared to studies with levels. Maternal ethnicity is related to both vitamin D concentrations and fetal growth patterns (36, 37). The study cohort was a multi-ethnic sample in the city of Rotterdam, the Netherlands. As compared to the population distribution in the city of Rotterdam, the percentage of Europeans was higher whereas the percentage of Moroccans was lower (36). We used two approaches to explore the role of maternal ethnicity. All main analyses were first adjusted for maternal ethnicity. Second, we observed tendencies for similar associations when we restricted the analyses to Europeans only. However, not all effect estimates were significant, probably due to smaller numbers.

Unfortunately, we did not have enough numbers in the other ethnic subgroups, to perform ethnic specific analyses. The results of these additional analyses suggest that the associations of vitamin D concentrations with fetal outcomes are not explained by maternal ethnicity. Further studies are needed to explore differences in effect estimates between ethnic subgroups. Finally, although we performed adjustment for a large number of potential maternal confounders, residual confounding by other lifestyle factors, might still be present, as in any observational study. The causality for the associations of vitamin D with fetal developmental outcomes cannot be established from observational studies only. Therefore, future studies are needed to establish causal relationships.

Conclusion

Our findings suggest that second trimester low maternal 25(OH)D concentrations are associated with third trimester fetal grow restriction and with increased risks of preterm birth, low birth weight, and small-size for gestational age at birth. These associations were not restricted to the extremes, but tended to be present across the full spectrum of maternal 25(OH)D concentrations. Although the causality of the observed associations need to be further established, these findings support current strategies to increase 25(OH)D concentrations in pregnant women.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments
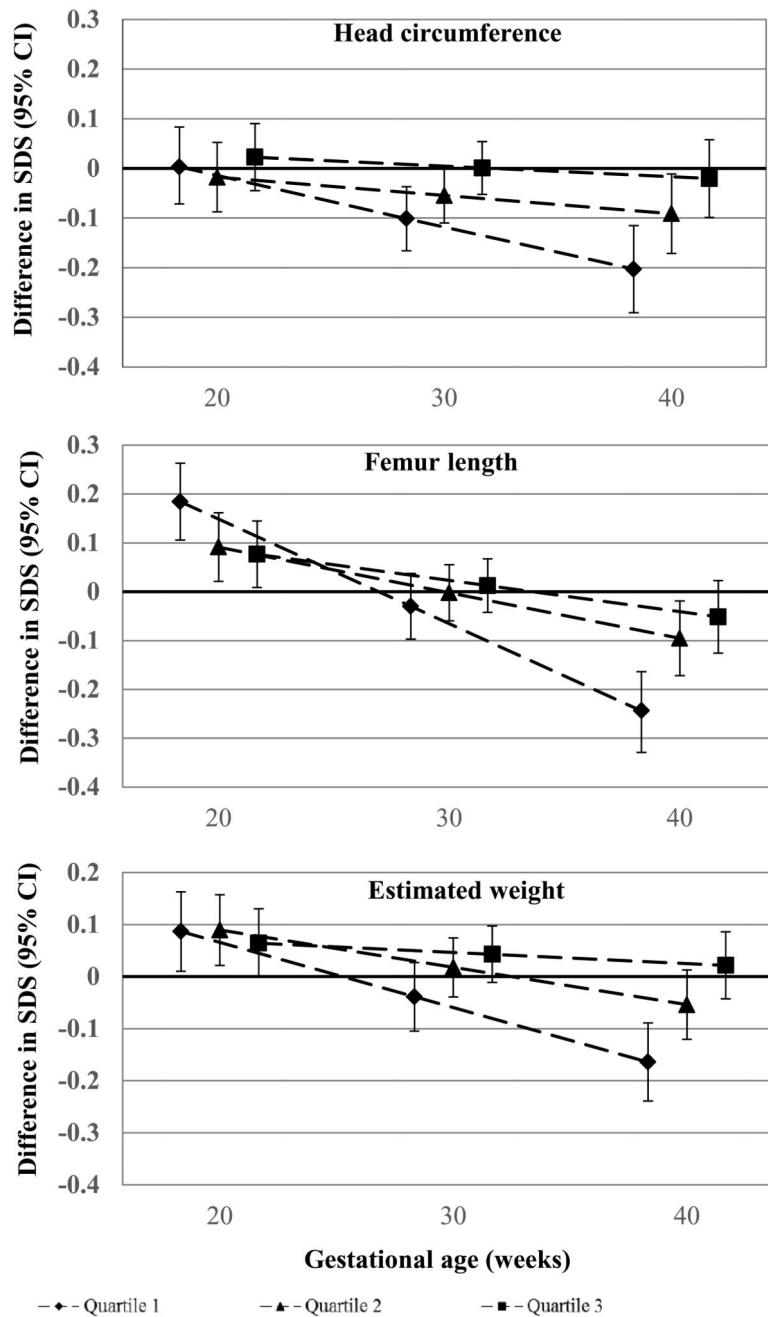
Abbreviations

CI   confidence interval

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Figure 1. Associations of Maternal Second Trimester 25(OH)D Concentrations with Fetal Growth Patterns (N = 7098)
Values are estimates based on repeated linear regression models and reflect the standard deviation score for each growth characteristic in offspring of mothers whose 25(OH)D concentrations during pregnancy were in the first, second and third quartile compared to offspring from mothers who had 25(OH)D concentrations in the fourth quartile. Length at birth represents the full body length and weight at birth is the measured birth weight.
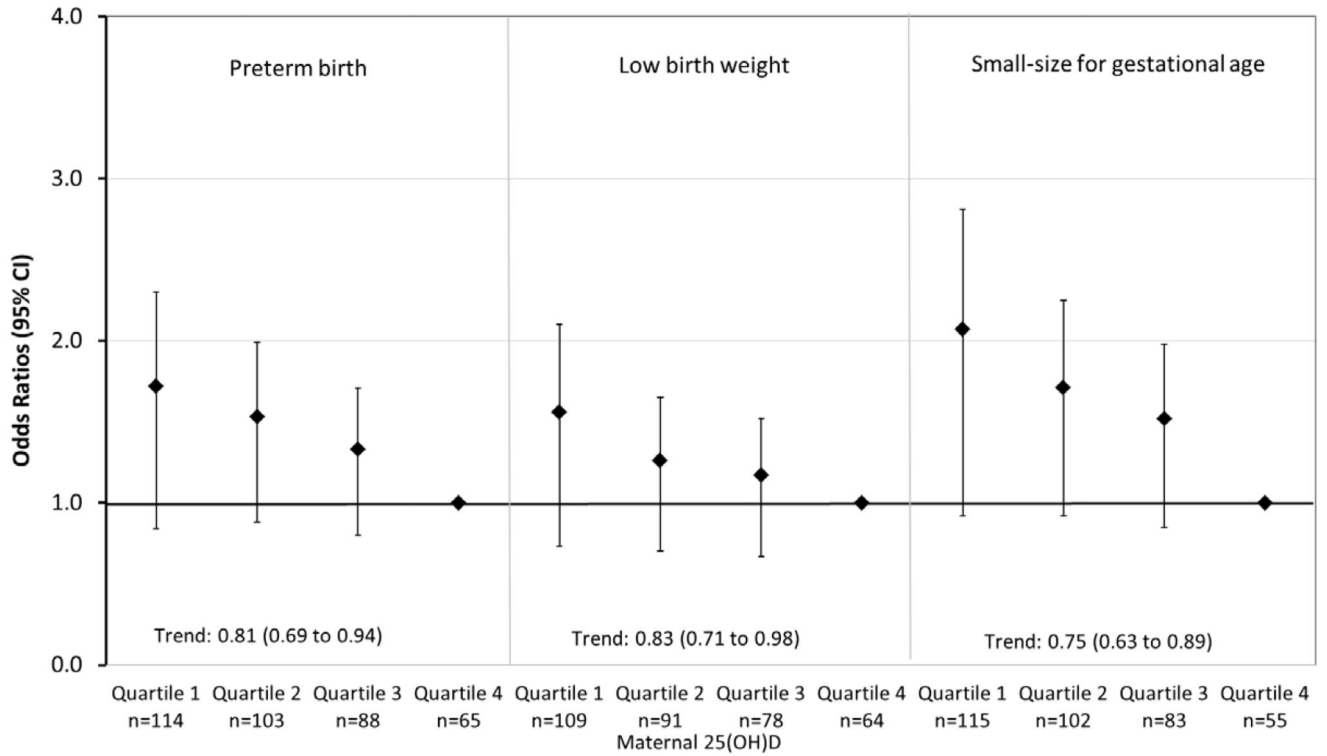
Figure 2. Associations of Maternal Second Trimester 25(OH)D Concentrations with the Risks of Adverse Birth Outcomes (N= 7098)

Values are logistic regression coefficients (95% confidence interval) and reflect the risk of adverse birth outcomes compared to the reference group. Continuous analyses reflect the risks of being preterm, having a low birth weight or being small-size for gestational age at birth per 1 SDS increase in maternal 25(OH)D. Multivariable model is adjusted for maternal characteristics (age, body mass index at intake, alcohol consumption, smoking during pregnancy, folic acid and vitamin supplements, energy, iron, zinc, and calcium dietary intake during pregnancy, education, ethnicity, gestational hypertensive disorders, gestational diabetes, parity, season when blood samples were drawn and the presence of anorexia).

Table 1

Subject Characteristics (N = 7098)[1]

Maternal characteristics	
Age, mean (SD), y	29.7 (5.2)
Body mass index at enrolment, median (95% range), kg/m^2	23.7 (18.7, 36.3)
Gestational age at enrollment, median (95% range), wk	13.9 (9.9, 22.9)
Nulliparous, No. (%)	3987 (56.2)
Education level, No. (%)	
- No higher education	4204 (59.2)
- Higher education	2894 (40.8)
Ethnicity, No. (%)	
- European	4069 (57.3)
- Cape Verdean	311 (4.4)
- Dutch Antillean	253 (3.5)
- Moroccan	471 (6.6)
- Surinamese	643 (9.1)
- Turkish	651 (9.2)
- Other	700 (9.9)
Presence of anorexia, No. (%)	
- No	6352 (89.5)
- Yes	503 (7.1)
- Maybe	243 (3.4)
Smoking during pregnancy, No. (%)	
- Never	5131 (72.3)
- Until pregnancy was known	665 (9.4)
- Continued	1302 (18.3)
Alcohol consumption during pregnancy, No. (%)	
- Never	3493 (49.2)
- Until pregnancy was known	974 (13.7)
- Continued	2631 (37.1)
Folic acid supplement use, No. (%)	
- No	2204 (31.0)
- Start in the first 10 weeks	2219 (31.3)
- Start periconceptional	2675 (37.7)
Vitamin supplement use, No. (%)	
- Yes	5049 (71.1)
- No	2049 (28.9)
Maternal energy intake (kcal)	2039 (490)
Maternal zink dietary intake (mg)	9.6 (1.6)
Maternal iron dietary intake (mg)	11.1 (2.1)
Maternal calcium dietary intake (mg)	1087 (418)
Maternal 25(OH)D concentrations, median (95% range), nmol/L	46.7 (7.0, 119.4)

- Severely deficient (< 25.0 nmol/L), No. (%)	1855 (26.1)
- Deficient (25.0 to 49.9 nmol/L), No. (%)	1919 (27.1)
- Sufficient (50.0 to 74.9 nmol/L), No. (%)	1619 (22.8)
- Optimal (75.0 nmol/L), No. (%)	1705 (24.0)
Season when maternal blood sample was take, No. (%)	
- Spring	2097 (29.5)
- Summer	1622 (22.9)
- Autumn	1702 (24.0)
- Winter	1677 (23.6)
Pregnancy complications, No. (%)	
- Gestational Hypertensive disorders	421 (5.9)
- Gestational Diabetes	67 (0.9)
Birth characteristics	
Female sex, No. (%)	3529 (49.7)
Preterm birth (<37 wk of gestation), No. (%)	370 (5.2)
Low birth weight (<2500 g), No. (%)	342 (4.8)
Small-size for gestational age at birth (<5th percentile), No. (%)	355 (5.0)
25(OH)D concentration in cord blood at birth, median (95% range), nmol/L	27.4 (4.7, 81.4)
Season when cord blood sample was taken, No. (%)	4.264
- Spring	1130 (26.5)
- Summer	1164 (27.2)
- Autumn	996 (23.4)
- Winter	977 (22.9)

[1]Values are percentages for categorical variables, means (SD) for continuous variables with a normal distribution, or medians (95% range) for continuous variables with a skewed distribution.

Table 2

Fetal Growth Characteristics (N = 7098)[1]

Second trimester (N = 7098)	
- Gestational age, median (95% range), wk	20.5 (18.6, 23.4)
- Head circumference, mean (SD), cm	17.9 (1.5)
- Femur length, mean (SD), mm	33.5 (3.6)
- Estimated fetal weight, mean (SD), g	382 (94)
Third trimester (N = 7018)	
- Gestational age at birth, median (95% range), wk	30.3 (28.4, 32.8)
- Head circumference, mean (SD), cm	28.5 (1.2)
- Femur length, mean (SD), mm	57.4 (3.0)
- Estimated fetal weight, mean (SD), g	1614 (253)
Birth (N = 7046)	
- Gestational age, median (95% range), wk	40.1 (35.6, 42.3)
- Head circumference, mean (SD), cm	33.8 (1.7)
- Body length, mean (SD), cm	50.2 (2.4)
- Weight, mean (SD), g	3414 (561)

[1]Values are means (SD) for continuous variables with a normal distribution, or medians (95% range) for continuous variables with a skewed distribution.

Table 3

Associations of Adverse Birth Outcomes with Cord Blood 25(OH)D Concentrations (N = 4262) [1]

Birth characteristics	N	Cord blood 25(OH)D (nmol/L)
Gestational age	**4262**	
<37.0 weeks	130	0.02 (-0.13, 0.17)
37.0-41.9 weeks	3849	Reference
42 weeks	283	-0.06 (-0.16, 0.05)
Trend		*-0.01 (-0.03, 0)*
Birth weight	**4258**	
<2000 grams	15	**-0.49 (-0.91, -0.06)**[2]
2000 - 2499 g	94	-0.01 (-0.18, 0.17)
2500 - 2999 g	607	-0.02 (-0.11, 0.06)
3000 - 3499 g	1535	Reference
3500 - 3999 g	1412	0.01 (-0.05, 0.07)
4000 - 4499 g	502	-0.01 (-0.10, 0.08)
4500 grams	93	0.06 (-0.12, 0.23)
Trend		***0.04 (0.01, 0.07)***[2,3]
Birth weight for gestational age	**4257**	
Small	164	-0.09 (-0.22, 0.05)
Normal	3881	Reference
Large	212	0.06 (-0.06, 0.18)
Trend		***0.03 (0.01, 0.06)***[2]

[1] Values are linear regression coefficients (95% confidence interval) and reflect the change in standard deviation (SDS) of cord blood 25(OH)D concentrations for each birth weight or gestational age group, compared to the reference group. Trend estimates represent the effect estimates for the continuous associations per SDS change in birth characteristics. Multivariable model is adjusted for fetal sex, maternal characteristics (age, body mass index at intake, alcohol consumption, smoking during pregnancy, folic acid and vitamin supplements, energy, iron, zinc and calcium dietary intake during pregnancy, education, ethnicity, gestational hypertensive disorders, gestational diabetes, parity and the presence of anorexia).

[2] *P value* < 0.05.

[3] Also significant after applying Bonferroni correction (*P value* <0.025).